

1/24

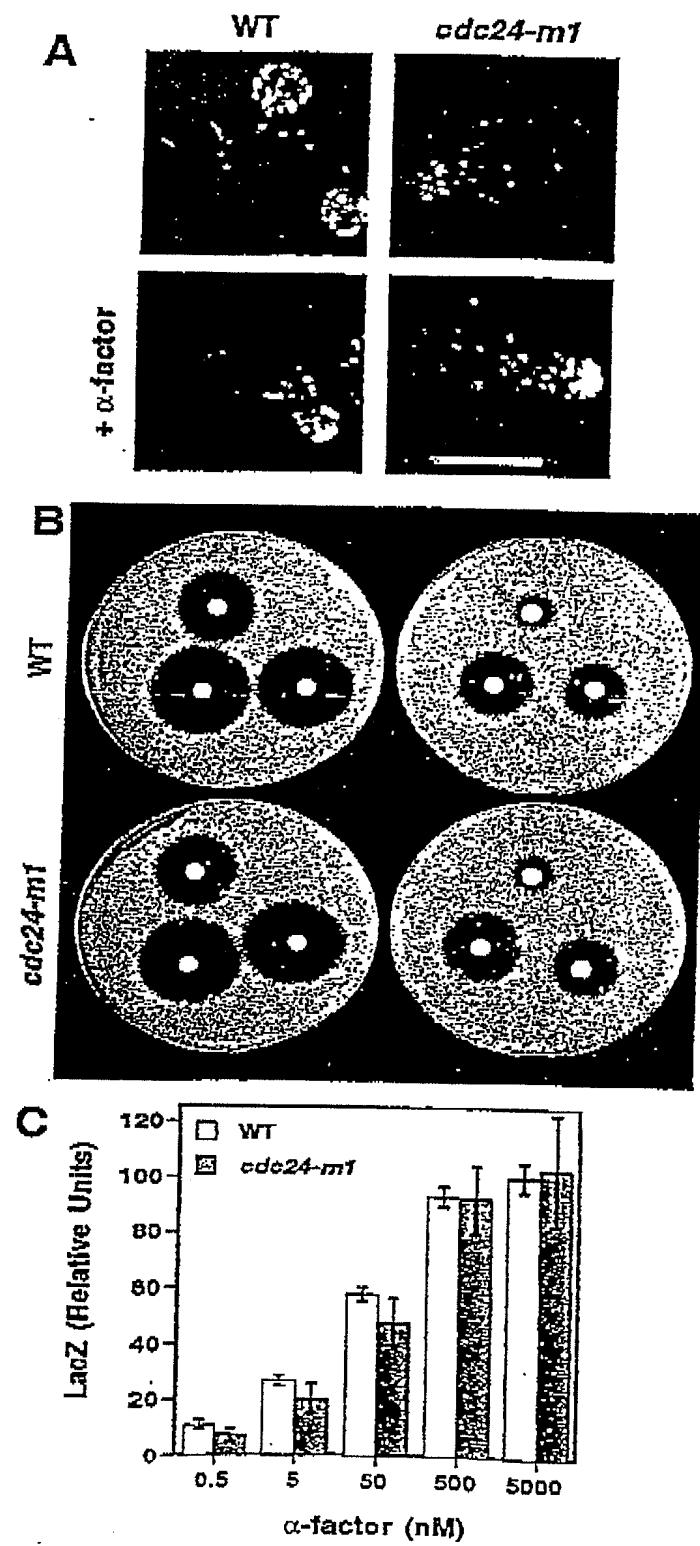


FIG. 1

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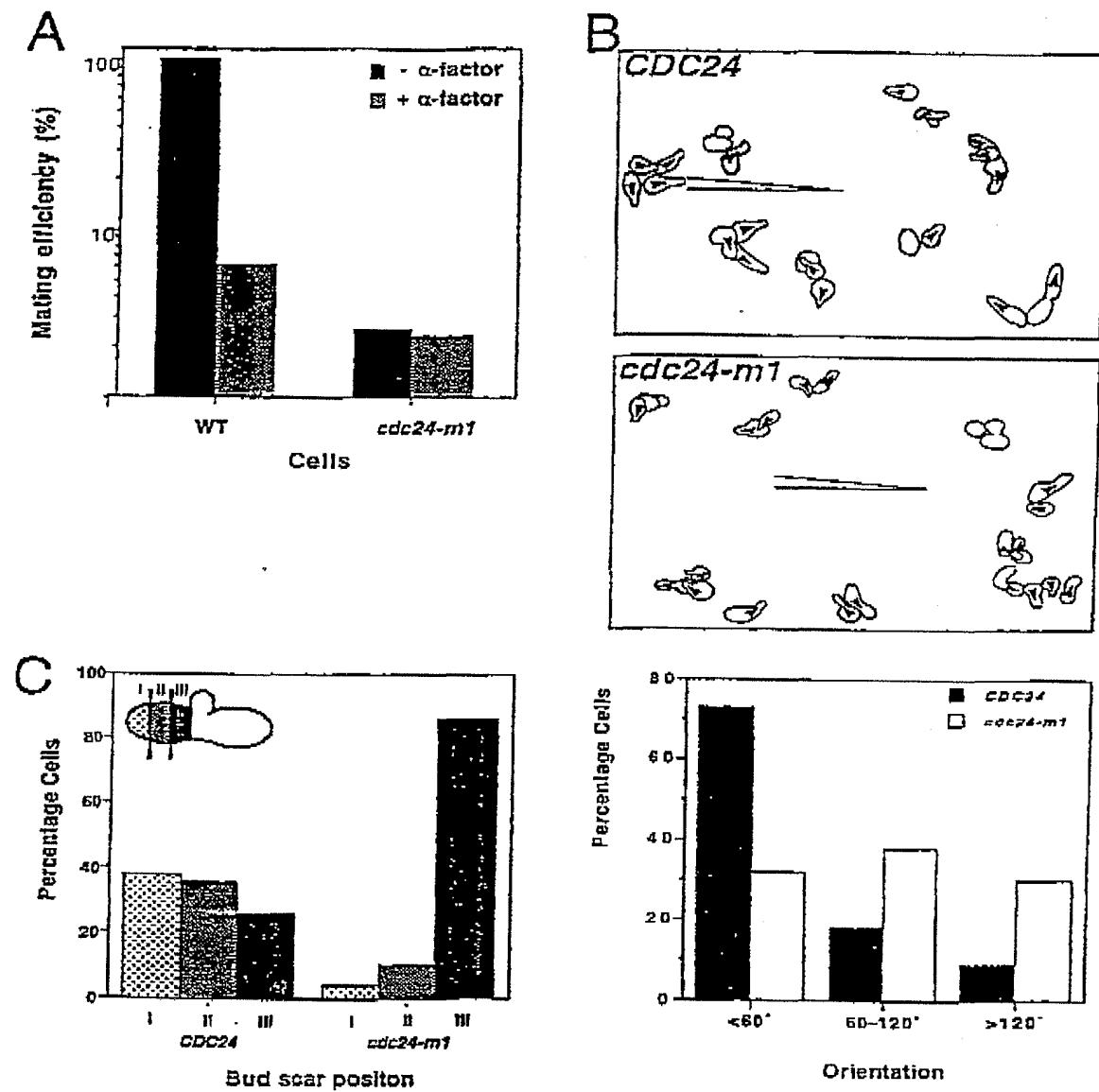
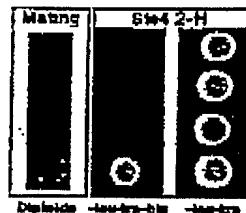


FIG. 2

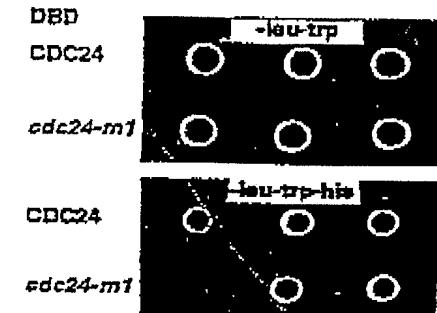
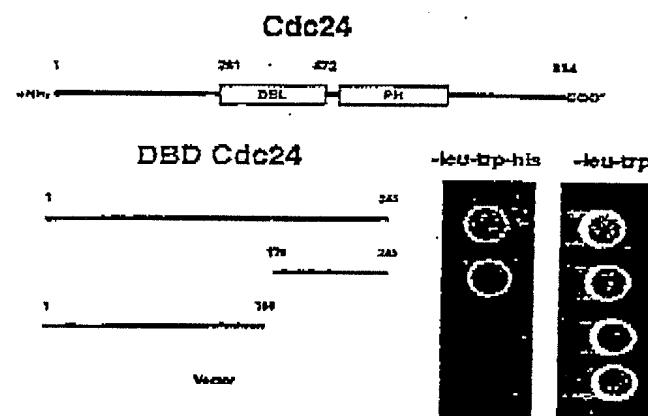
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A

Cdc24-m1	QFKLPVIAYDDLRVCKKSI
Cdc24-m2	QFKLPVIAASGLRVCKKSI
Cdc24-m3	QFKLPVIAZDGLRVCKKSI
Cdc24 Sc 181	QFKLPVIAASDGLRVCKKSI 189
+ + - + +	
Dm Hu 385	QYEDDVILSPELKVQHSTI 403

**B**

AD STE4 CDC42 BEM1

**C****D**

GST
 GSTCdc24 GST
 Extracts Eluates

FIG. 3

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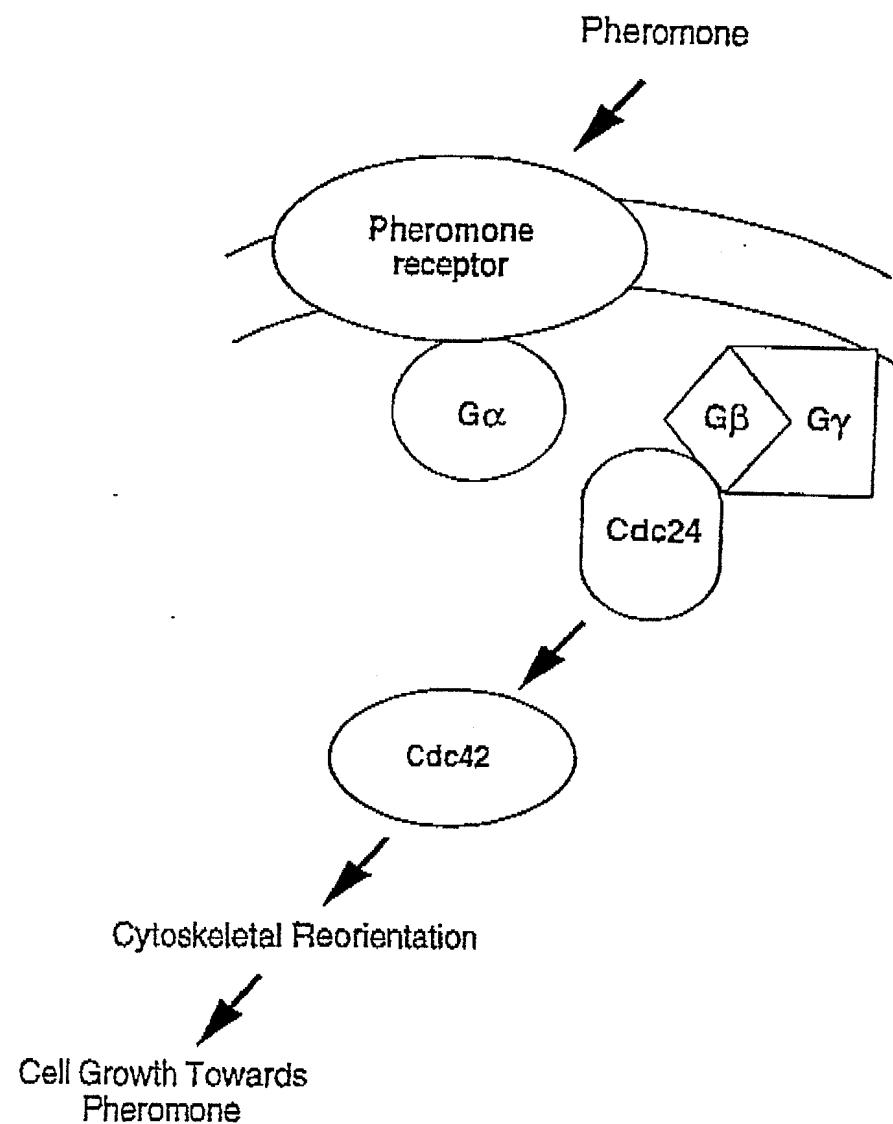


FIG. 4

Figure 5

5	2113/1	2143/11
	ATG GAA CAT CCA CCA GCA GCT CTC AGA ACA	TTT TCA ACC CAA TCA ACT TCA TCT TTG AAT
	M E H P P A A L R T	F S T Q S T S S L N
10	2173/21	2203/31
	TCA GTA AGT ACT GTT TCG TCT TCA AGA ATT	GTT TCT CTG GGC CCA GTC AAT ATA AAC AAT
	S V S T V S S R I	V S S G P V N I N N
	2233/41	2263/51
	TTC AAT AAA CCA AGT ACT CCC AAA GAC CAT	TTA TTC TAT CGA TGT GAA TCA CTA AAA CGA
	F N K P S T F K D H	L F Y R C E S L K R
15	2293/61	2323/71
	AAA CTA CAA AAA ATC CCT GGC ATG GAA CCA	TTT TTG AAC CAA GCT TTC AAT CAG GCT GAA
	K L Q K I P G M E P	F L N Q A F N Q A E
	2353/81	2383/91
	CAA CTC AGT GAA CAA CAA GCA TTG GCT TTG	GCA CAG GAA AGA AGC AAT GGA AAT GGA CAT
20	Q L S E Q Q A L A L	A Q E R S N G N G H
	2413/101	2443/111
	AGT AAT GGC AAA CGT CAT CAA TCA TTA GAC	GGT GCC ATG AAT AGA CTT TCA GTT GGT TCT
	S N G K R H Q S L D	G A M N R L S V G S
	2473/121	2503/131
	GAT AGT AGT TCG ATC CAA GGT TCA TTG ACA	CGA ATG GCC ACC AAT GCG TCA ACG TCA TCT
	D S S S I Q G S L T	R M A T N A S T S S
	2533/141	2563/151
	TTA ATC AGT GGT ATG CCA AAC AAC AAC ACT	TTA TTT ACG TTT ACT GCA GGG GTT TTA CCA
	L I S G M P N N N T	L F T F T A G V L P
30	2593/161	2623/171
	GCT AAT ATT AGT GTC GAT CCT GCT ACC CNT	CTT TGG AAA TTG TTC CAA CAA GGG GCC CCC
	A N I S V D P A T H	L W K L F Q Q G A P
	2653/181	2683/191
	TTT TGT GTT CTT ATC AAT CAT ATC CTT CCT	GAT TCC CAA ATA CCA GTT GTC AGT TCT GAT
	F C V L I N H I L P	D S Q I P V V S S D
	2713/201	2743/211
	GAC TTG AGA ATT TGC AAA AAA TCA GTA TAT	GAC TTT TTA ATT GCC GTC AAG ACA CAA TTG
	D L R I C K K S V Y	D F L I A V K T Q L
40	2773/221	2803/231
	AAT TTT GAT GAC GAG AAT ATG TTC ACT ATA	TCC AAT GTT TTC TCC GAC AAT GCC CAA GAT
	N F D D E N M F T I	S N V F S D N A Q D
	2833/241	2863/251
	TTA ATC AAG ATT ATT GAT GTC ATT AAT AAA	CTA CTT GCT GAG TAC TCA GAT GCT AGT GAC
	L I K I I D V I N K	L L A E Y S D A S D
45	2893/261	2923/271
	CTG GGT GGT GGC GAT GAA GAT GTA AAT ATG	GAT GTT CAA ATT ACC GAT GAA AGA TCA AAA
	S G G G D E D V N M	D V Q I T D E R S K
	2953/281	2983/291
	GTT TTC CGA GAA ATT ATC GAA ACA GAA AGA	AAA TAT GTT CAA GAC TTG GAA CTA ATG TGT
50	V F R E I I E T E R	K Y V Q D L E L M C
	3013/301	3043/311
	AAA TAC CGT CAA GAT CTA ATT GAA GCC GAA	AAT TTG TCT TCA GAA CAA ATT CAC TTG TTA
	K Y R Q D L I E A E	N L S S E Q I H L L
	3073/321	3103/331
	TTC CCA AAT TTA AAT GAG ATT ATT GAT TTT	CAA AGA CGA TTC CTC AAT GGG TTA GAA TGT
	F P N L N E I I D F	Q R R F L N G L E C
	3133/341	3163/351
	AAC ATC AAT GTA CCT ATT AGA TAT CAA AGA	ATT GGA TCA GTA TTT ATT CAT GCT TCT TTG
	N I N V P I R Y Q R	I G S V F I H A S L
60	3193/361	3223/371
	GGC CCT TTC AAT GCT TAT GAA CCT TGG ACT	ATA GGA CAA TTG ACG GCG ATT GAT TTG ATC
	G P P N A Y E P W T	I G Q L T A I D L I
	3253/381	3283/391
	AAC AAA GAA GCT GCT AAT TTG AAA AAA TCG	TCA AGT CTA CTT GAT CCT GGG TTT GAA CTT
65	N K E A A N L K K S	S S L L D P G F E L
	3313/401	3343/411
	CAA TCG TAT ATA TTA AAG CCG ATC CAA AGA	TTG TGT AAA TAC CCA CTT TTG TTG AAA GAG
	Q S Y I L K P I O R	L C K Y P L L L K E
	3373/421	3403/431
70	TTA ATC AAA ACA TCA CCA GAA TAT TCA AAA	CAG GAC CCC CAT GGC AGC TCG TCA TCG ACA
	L I K T S P E Y S K	Q D P H G S S S S T
	3433/441	3463/451
	TCA TTC AAT GAA TTA TTG GTG GCT AAA ACT	GCA ATG AAA GAA TTG GCA AAT CAA GTC AAT
	S F N E L L V A K T	A M K E L A N Q V N

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3493/461
 GAG GCG CAA AGA CGA GCA GAA AAT ATC GAA CAT TTG GAA AAA CTA AAA GAA AGA GTA CGT
 E A Q R R A E N I E H L E K L K E R V G
 3553/481
 5 AAT TGG CGT GGG TTT AAT TTG GAT GCT CAA GGA GAA CTA TTA TTC CAC GGA CAA GTT GGG
 N W R G F N L D A Q G E L L F H G Q V G
 3613/501
 GTT AAA GAT GCT GAA AAT GAA AAG GAA TAC GTT GCT TAT CTT TTT GAA AAA ATC GTA TTT
 V K D A E N E K E Y V A Y L F E K I V F
 10 3673/521
 TTT TTC ACA GAA ATT GAT GAT ACC AAA AAA TCT GAT AAA CAG GAA AAG AAG AGC AAG TTT
 F F T E I D D T K K S D K Q E K K S K F
 3733/541
 15 TCG ACA AGA AAG AGA TCA ACT TCA TCA AAT CTT AGT TCA TCG ACT ACT AAT TTG TTG GAA
 S T R K R S T S S N L S S S T T N L L E
 3793/561
 TCA ATA AAC AAT TCC CGA AAG GAT AAC ACA TTG CCA TTG GAA TTA AAG GGA AGA GTT TAT
 S I N N S R K D N T L P L E L K G R V Y
 3853/581
 20 ATA TCG GAG ATT TAT AAC ATT TCC GCA CCA AAC ACT CCT GGC TCA ACT CTA ATC ATC TCA
 I S E I Y N I S A P N T P G S T L I I S
 3913/601
 TGG TCA GGT AGA AAG GAA AGC CGC TCA TTC ACT TTG AGA TAT CGT AGT GAA GAA GCC AGA
 W S G R K E S G S F T L R Y R S E E A R
 3973/621
 AAC CAA TGG GAA AAG TGT TTA CGT GAT TTG AAG ACT AAT GAA ATG AAT AAA CAA ATT CAT
 N Q W E K C L R D L K T N E M N K Q I H
 4033/641
 25 AAG AAG TTA CGT GAT TCC GAC CTG TCA TTT AAT ACT GAT GAC TCT GCC ATA TAT GAT TAC
 K K L R D S D S S F N T D D S A I Y D Y
 4093/661
 30 ACG GGT ATT AGT ACG TCA CCA GTC AAT CAA TCA ACT CAA CAA CAA TAC TAT GAT CAT CGG
 T G I S T S P V N Q S T Q Q Y Y D H R
 4153/681
 35 GGC TCT CAC AGT TCC CGC CAT CAC TCA TCG TCA TCC ACT TTG AGT ATG ATG AAG AAT AAT
 G S H S S R H H S S S S T L S M M K N N
 4213/701
 AGA GTT AAA TCT GGT GAT TTG AGT AGA ATA TCT TCA ACT TCA ACA ACA TTA GAT TCT TTC
 R V K S G D L S R I S S T S T T L D S F
 40
 4273/721
 AGT AAC AAC TTG AAT CGG TCA CCA AAT ACC ACT AAT CCA TCT TTG ATG TCT TCA GAT GCC
 S N N L N G S P N T T N P S L M S S D A
 4333/741
 45 ACC AAA ACA ATT CCA ACA TTT GAC GTT GCA ATT AAA TTG CTT TAC AAA TCG ACA GAA TTG
 T K T I P T F D V A I K L L Y K S T E L
 4393/761
 TCA GAG CCA TTG ATT GTC AAT GCA CAA ATT GAG TAT AAT GAC CTT TTA CAG AAA ATT ATC
 S E P L I V N A Q I E Y N D L L Q K I I
 4453/781
 50 TCC CAG ATT ATC ACT TCG AAC TTG GTG GCA GAT GAT GTC AAT ATT AGT CGA TTG AGA TAT
 S Q I I T S N L V A D D V N I S R L R Y
 4513/801
 AAA GAC GAC GAA GGA GAC TTT GTG AAT TTG AAT TCA GAT GAT GAT TGG GGG TTA GTG CTT
 K D D E G D F V N L N S D D D W G L V L
 55 4573/821
 GAT ATG TTA ACC AGT GAA GAC TTT TAC CAA ACA TCA AGC AAT GAA AAA CGA CTG GTG ACA
 D M L T S E D F Y Q T S S N E K R S V T
 4633/841
 GTG TGG GTT TCT TGA
 60 V W V S *

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Figure 6

5 Blastp line up of S.c. Cdc24p and C.a. Cdc24p

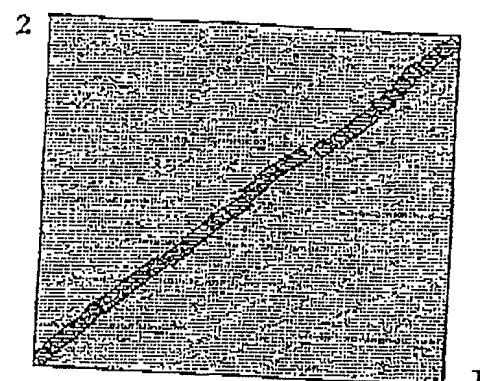
	S.c. Cdc24p: 1	MAIQ---TR-FA M R F+	8
10	C.a. Cdc24p: 1	MEHPPAALRTFSTQ	14
	S.c. Cdc24p: 9	SGTSLSDLKPKPATSISIPMQNV--MNKPVTEQDSLHFICANIRKRLLEVLPQLKPFLQL 66 S +SL+ + S+ +S N+ NKP T +D LF+ C +++++L+ +P ++PFL	
	C.a. Cdc24p: 15	STSSLNSVSTVSSSIVSSGPVNINNFNKPSTPKDHLFYRCESLRKRKLQKIPGMEPFLNQ 74	
15	S.c. Cdc24p: 67	AYQSSEVLSERQSLLLSQKQHQELLKSGNGANRDSSLAP---TLRSSSISTATSLMSMEG 123 A+ +E LSE+Q+L L+Q++ SNG S D A ++ S S S SL M	
	C.a. Cdc24p: 75	AFNQAEQELSEQQALALAQERSNGNGHSNGKRHQSLGAMNRLSVDGSDDSSIQGSLTRMAT 134	
20	S.c. Cdc24p: 124	ISYTNSNFSATPNMEDTLLTFSMGLPITMDCDFVTQLSOLFOOGAPLCLIRFNSVKPQFK 183 + T+S S PN +TL TF+ G+LP + DP T L +LFQQGAP C+L N + P +	
	C.a. Cdc24p: 135	NASTSSLISGMNPN-NNTLPTFTAGVLPANTSVDPATHLWKLFFQGAPFCVCLINHILPDSQ 193	
	S.c. Cdc24p: 184	LPVIASDDLKVCKKSIYDFILGCKKHFAFMDEELFTISDVFANSTSOLVKLEVETLMN 243 +PV++SDDL++CKKS+YDF++ K F+DE +FTIS+VF+++ L+K++V+ L+	
25	C.a. Cdc24p: 194	IPVVSSDDLRLICKKSVYDFLIAVKTQLNFDDENMFTISNVFSDNAQDLIKIIDVINKLLA 253	
	S.c. Cdc24p: 244	SSPTIFPSKSKTQQIMNAENQHRHQPQQSSKKHNEYVKIIKEFVATERKYVHDLEILDKY 303 S + + + + E K+ +E + TERKYV DLE++ KY	
	C.a. Cdc24p: 254	EYSDASDGGGDEDV-----NMDVQITDERSKVFREIIETERKYVQDLELMCKY 302	
30	S.c. Cdc24p: 304	RQQLLDSNLITSEELYMLFPNLGDAIDFQRRFLISLEINALVEPSKQORIGALFMH-SKHF 362 RQ L+++ ++SE++++LFPNL + IDFQRRFL LE N V QRIG++F+H S	
	C.a. Cdc24p: 303	RQDLIEAENLSSEQIHLFFPNLNEIIDFQRRFLNGLECNINVPIRYQRIGSVFIHASLGP 362	
35	S.c. Cdc24p: 363	FKLYEPWSIGQNAIEFLSSTLHKMRVDESQRFIINNKLELQSFYKPVQRLCRYPLLVK 422 F YEPW+IGQ AI+ ++ ++ S +++ + ELOQ+ + KP+QRLC+YPLL+K	
	C.a. Cdc24p: 363	FNAYEPWTIGQLTAIDLINKEAANLKKSSS---LLDPGFELOQSYILKPIQRLCKYPLLK 419	
40	S.c. Cdc24p: 423	ELLAE-----SSDDNNNTKELEAALDISKNIARSINENQRRTENHQVVKLYGRV 471 EL+ SS + EL A K +A +NE QR EN + ++KL RV	
	C.a. Cdc24p: 420	ELIKTSPEYSKQDPHGSSSSTSFNELLVAKTAMKELANQVNEAQRRAENIEHLEKLKERV 479	
	S.c. Cdc24p: 472	VNWKGYRISKFGELLYFDKVFISTTNSSSEPEREFEVYLFEKIIILFSEVVTKKSASSLI 531 NW+G+ + GELL+ +V + +E E+E+ YLFEKI+ F+E+ K+	
45	C.a. Cdc24p: 480	GNWRGFNLLDAQGELLFHGQVGV---KDAENEKEYVAYLFEKIVFFTEIDDTKKSQDKQE 535	
	S.c. Cdc24p: 532	LKKKSSTSASISASNITDNNGSPHHSYHKRHSNSSSSNNIHLSSSSAAIIHSSTNSSDN 591 K K ST ++SN+ SSS ++ S NS +	
	C.a. Cdc24p: 536	KKSFKSTRKRSTSSNL-----SSSTTNLLESINNSRKD 568	
50	S.c. Cdc24p: 592	NSNNSSSSSLFKLSANEPKLSDLRGRIMIMNLNQIIPQN--NRSLNITWESIKEQGNFLK 649 N+ L+L+GR+ I + I N +L I+W KE G+F L+	
	C.a. Cdc24p: 569	NT-----LPLELKGGRVYISEIYNISAPNTPGSTLIIWSGRKESGSFTLR 613	
55	S.c. Cdc24p: 650	FKNEBTRDNWSSCLQQLIHDLKNEQFKARHHSTSTTSS-----TAKSSSMMSPPTT 701 ++EE R+ W CL+ L + N+Q + S S+ ++ T S+S ++ +T	
	C.a. Cdc24p: 614	YRSEEARNQWEKCLDLKTNEMNKQIHKKLRSDSSFNTDDSAIYDYGISTSPVNQSTQ 673	
60	S.c. Cdc24p: 702	MNTPNHHNSRQT--HDSMASFSSSHMKRVS---DVLPKRRRTTSSSEPESEIKS----- 748 +H S + H S ++ S RV + TT SF ++	
	C.a. Cdc24p: 674	QQYYDHRGSHSSRHSSSSSTLSMMKNNRVKSGDLSRISSTTLDGSFSNNLNGSPNTTNP 733	
65	S.c. Cdc24p: 749	--ISENPKNSIPESSILFRISYNNNSNNNTSSSEIFTLLVEKVNFPDDLIMAINSKI--SN 804 +S + +IP + ++ Y +T SE L+V ++DL+ I S+I SN	
	C.a. Cdc24p: 734	SLMSSDATKTIPTPDVAIKLLY---KSTELSE--PLIVNAQIEYNDLLQKIIISQIITSN 787	
	S.c. Cdc24p: 805	THNNNISPITKIKYQDEDGDFVVLGSDEDWNVAKEMLAENNEKFLNIRLY 854 ++++ I++++Y+D++GDFV L SD+DW + +ML + F +	
	C.a. Cdc24p: 788	LVADDVN-ISRLRYKDDEGDFVNLNSSDDWGLVLDMLTSED--FVOTSSNEKRSVTWVWS 844	

Figure 7a

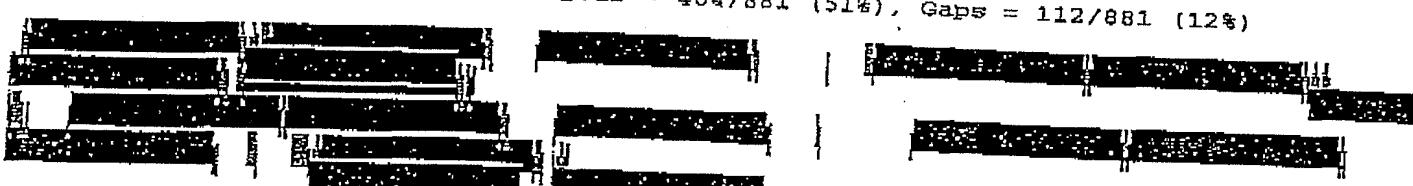
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BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.0.9 [May-07-1999]

Matrix **Q-BLOSUM62** gap open: **11** gap extension: **1**
 x_dropoff: **50** expect: **10.0** wordsize: **3** Filter Align

Sequence 1 **Icl|S.c. Cdc24p Length 854 (1 .. 854)**Sequence 2 **Icl|C.a. Cdc24p Length 844 (1 .. 844)**

NOTE: The statistics (bitscore and expect value) is calculated based on the size of nr database
 Score = 446 bits (1136), Expect = e-124
 Identities = 288/881 (32%), Positives = 464/881 (51%), Gaps = 112/881 (12%)



Query: 9 SGTSLSDLKPCKPSATSISIPMQNV--MNKPVTEQDSLPHICANIRKRLVELPQLKPFLQL 66
 S +SL+ + S+ +S N+ NKP T +D LF+ C +++++L+ +P ++PFL
 Sbjct: 15 STSSILNSVSTVSSSRIVSSGPVNINNNFKPSTPKDHLFYRCESLKRKLQKIPGMEPFLNQ 74

Query: 67 AYQSSEVLSERQSLLLSQKQHQELLKSGNGANRDSSDLAP---TLRSSSISTATSLMSMEG 123
 A+ +E LSE+Q+L L+Q++ SNG S D A ++ S S S SL M
 Sbjct: 75 AFNQAEQLSEQQALALAQERSNGNGHSNGKRHQLDGAMNRLSVDGSDSSSIQGSLTRMAT 134

Query: 124 ISYTNSNPSAT?NMEDTLLTFSMGILPITMDCDPVTQLSQLFQQGAPLCILFNSVKPQFK 183
 + T+S S PN +TL TF+ G+LP + DP T L +LFQQGAP C+L N + P +
 Sbjct: 135 NASTSSLISGMPN-NNTLFTFTAGVLPANISVDPATHLWKLQQGAPFCVLINEHILPDSQ 193

Query: 184 LPVIASDDLKVCKKSITYDFILGCKKHFAFNDEELFTISDVFANSTSOLVKVLEVWETLMN 243
 +PV++SDDL++CKKS+YDF++ K F+DE +FTIS+VP+++ L+K+++V+ L+
 Sbjct: 194 IPVVSSDDLICKKSVDLIAVKTQLNPDDENMFTISNVFSDNAQDLIKIIDVINKLLA 253

Query: 244 SSPTIFPSKSKTQQIMNAENQERHQPOQQSSKKHNEYVKIIKEFVATERKVVHDLEILDKY 303
 S + + + + E K+ +E + TERKVV DLE++ KY
 Sbjct: 254 EYSDASDGGGDEDV-----NMDVQITDERSKVFREIIETERKVVQDLEMCKY 302

Query: 304 RQQLLDSNLITSEELYMLF?NLGDAIDFQRRFLISLEINALVEPSKQRIGALFMH-SKHF 362
 RQ L++- ++SE++++LFPNL + IDFQRRFL LE N V QRIG++F+H S
 Sbjct: 303 RQDLTEAENLSSSEQIHLLFPNLNEIIDFQRRFLNGLECNINVPIRYQRIGSVFIHASLGP 362

Query: 363 FKLYEPWSIGQNAAJEFLSSTLHKMRVDESQRFIINNNKLELQSFLYKPVQRLCRYPLLVK 422
 F YEPW+IGQ AI+ ++ ++ S +++ ELQS+ KP+QRLC+YPLL+K
 Sbjct: 363 FNAYEPWTIGQLTAIDLINKEANLKKSSS---LLDPGFFTGSVTKPDKP

Figure 7a cont.

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Query: 423 ELLAE-----SSDDNNNTKELEAALDIKNIARSINENQRTENQVVKLYGRV 471
 EL+ SS + EL A K +A +NE QRR EN + ++KL RV

Sbjct: 420 ELIKTSPEYSKQDPHGSSSSTSFNELLVAKTAMKELANQVNEAQRAENIEHLEKLKERV 479

Query: 472 VNWKGYRISKFGELLYFDKFVISTTNSSEPEREFEVYLFEKIIILFSEVVTKKSASSLI 531
 NW+G+ + GELL+ +V+ +E E+E+ YLFKEKI+ F+E+ K+

Sbjct: 480 GNWRGFNLDAQGELLFHGQVGV---KDAENEKEYVAYLFEKIVFFFTEIDDTKKSQDKQE 535

Query: 532 LKKKSSTSASISASNITDNNGSPHYSYHKRHSNSSSSNNIHLSSSSAAATIHSSTNSSDN 591
 K K ST ++SN+ SSS ++ S NS +

Sbjct: 536 KKSKESTRKRSTSSNL-----SSSTTNLLESINNSRKO 568

Query: 592 NSNNSSSSSLFKLSANEPKLDLGRIMIMNINQIIPQN--NRSLNITWESIKEQGNFLK 649
 N+ L+L+GR+ I + I N +L I+W KE G+F L+

Sbjct: 569 NT-----LPLELKGRVYISEIYNISAPNTPGSTLITISWSGRKESGSPTLR 613

Query: 650 FKNEETRDNWSSCLQQLIHDLKNEQFKARIHSSTTSS-----TAKSSSSMMSPTTT 701
 +++EE R+ W CL+ L + N+Q + S S+ ++ T S+S ++ +T

Sbjct: 614 VRSEEARQWEKCLRDLKTNEMNKQIHKKLRSDDSSNTDDSAIYDYTGISTSPVNQSTQ 673

Query: 702 MNTPNHHNSRQT--HDSMASFSSSHMKRVS---DVLPKRRRTSSSEFESEIKS----- 748
 +H S + H S ++ S RV + TT SF + +

Sbjct: 674 QQYYDHRGSHSSRHSSSSTLSMMKNNRVKSGDLSRISSTSTLDSPSNLNGSPNTTNP 733

Query: 749 --ISENFKNSTIPESSILFRIYSNNNSNNTSSSEIFTLLVEKVNWFDDLIMAINSKI--SN 804
 +S + +IP + ++ Y +T SE L+V ++DL+ I S+I SN

Sbjct: 734 SLMSSDATKTIPTFDVAIKLLY---KSTELSE--PLIVNAQIEYNDLLQKIIISQITTSN 787

Query: 805 THNNNISPITKIKYQDEDGDFVVLGSDEDWNVAKEMLAENN 845
 +++ I++++Y+D++GDFV L SD+DW + +ML +

Sbjct: 788 LVADDVN-ISRLRYKDDEGDFVNLSDDDHWGLVLDMLTSED 827

CPU time: 0.26 user secs. 0.02 sys. secs 0.28 total secs.

Gapped Lambda K H
 0.270 0.0470 0.230

Matrix: BLOSUM62

Gap Penalties: Existence: 11, Extension: 1

Number of Hits to DB: 12253

Number of Sequences: 0

Number of extensions: 709

Number of successful extensions: 15

Number of sequences better than 10.0: 1

Number of HSP's better than 10.0 without gapping: 1

Number of HSP's successfully gapped in prelim test: 0

Number of HSP's that attempted gapping in prelim test: 0

Number of HSP's gapped (non-prelim): 1

length of query: 844

length of database: 90,077,593

effective HSP length: 63

effective length of query: 781

effective length of database: 83353792

effective search space: 65099311552

effective search space used: 65099311552

T: 9

A: 40

X1: 16 (7.2 bits)

X2: 128 (49.9 bits)

X3: 128 (49.9 bits)

S1: 42 (21.9 bits)

S2: 73 (32.8 bits)

Figure 7b

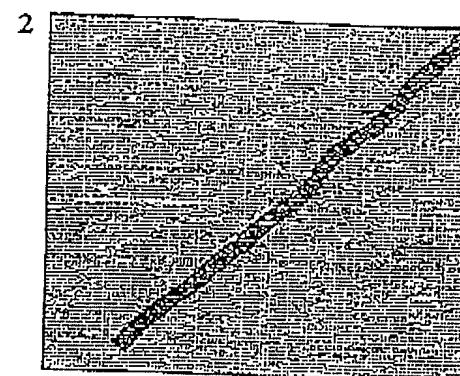
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BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.0.9 [May-07-1999]

Matrix **BLOSUM62** gap open: **11** gap extension: **1**
 x_dropoff: **50** expect: **10.0** wordsize: **3** Filter **Off** Align

Sequence 1 lcl|S.c. Cdc24p Length 854 (1..854)

Sequence 2 lcl|S.p. Cdc24p Length 834 (1..834)



NOTE: The statistics (bitscore and expect value) is calculated based on the size of nr database

Score = 238 bits (601), Expect = 1e-61
 Identities = 212/760 (27%), Positives = 348/760 (44%), Gaps = 94/760 (12%)



Query: 156 DPVTQLSQLFQQGAPLCILFNSVKPQPKLPVIAASDDLK---VCKKS1YDFILGCKKHFAF 212
 DPVT++ + G PLC LEN + + KL V + S L+ VCK S+Y F+L CK
 Sbjct: 67 DPVTEIWLFCLRGYPLCALFNCLPVKQKLEVNSVSLENTINVCKASLYRFMLMCKNELGL 126

Query: 213 NDEELFTISDVFANSTSSQLVKVLEVETLMNSSPTIFPSKS5KTQQIMNAENQHRHQPOQS 272
 D LF+IS+++ ST+ LV+ L+ +2 L+ +KS + + ++ S
 Sbjct: 127 TDAALFSISEIYKPSTAPLVRALQTIELLKKYEVNTKSSSTPSPSTDDNVPTGTLNS 186

Query: 273 SKKHNEYVKITKEFVATERKYVHDLEILDKYRQQLLDSNLITSEELYMLFPNLGDAIDFQ 332
 ++ E TE KY+ DLE L Y L + + + + + F NL + +DFQ
 Sbjct: 187 LIASGR--RVTAELVETELKYIQLDELYLSNYMVLQQKQILSQDTILSIFTNLNEILDFO 244

Query: 333 RRFLISLEINALVEPSKQRIGALFMHSKHFKLYEPWSIG-QNAATIFLSSTLHKMRVDE 391
 RRFL+ LE+N + +QR+GALF+ + F +Y+ + .NA + + ++V
 Sbjct: 245 RRFLVGLEMNLSLPVEEQRILCALFIALEEGFSVYQVFCTNFPNAQQLIIDNQNQLLKVAN 304

Query: 392 SQRFTINNKLELQSFPLYKPVQRLCRYPLLVKELL-AE55DDNNNTKEIPLAALDISKNTIARS 450
 ++ EL + L KP+QR+C+YPLL+ +LL S +EL+ + +A
 Sbjct: 305 ---LLEPSYELPALLLIKPIQRICKYPLLNLQQLKGTPSGYQYEEEELKQGMACVVVRVANQ 360

Query: 451 INENQRRTENHQVVKLYGRVNNWKGYRISKFGELLYFDKVP1STNTNSSEPEREFEVYL 510
 +NE +R EN + +L RV++WKGY + FG+LL +D V + ++ ERE+ VYL
 Sbjct: 361 VNETRRIHENRNAAIELEQRVIDWKGYSLQYFGQLLVWDVVNV---CKADIEREYHVYL 416

Query: 511 FEKIIILFSEVVT-KKSASSLILKKKSSTSAS-----ISASNITDN----- 550
 FEKI++ E+ T K+ A S+ + KK+ S I SNIT
 Sbjct: 417 FEKILLCCKEMSTLKRQARSISMNKKTKRLDSLOLKGRPI TSNTT

Figure 7b cont..

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Query: 551 --NGSPHHSYHKRHSNSSSNHLS-----SSAAATIHSSTNSSDNNNSNNSSS 599
 G P H + S+ + +S + I S+ ++ N N SSS
 Sbjct: 477 FWRGDPQHESFILKLRNEESHKLWMSVLNRLLWKNEHGSFKDIRSAASTPANPVYNRSSS 536

Query: 600 SLFKLSANEPKLD-LRGRIMIMNLN---QIIPQNNRSLNITWESIKEQGNFLLKFKNEET 655
 K N D LR + N+N I + + +S T + K+ K+ T
 Sbjct: 537 QTSK-GYNSSDYDILLRTHSLDENVNSPTSISSPSSKSSPFTKTTSKDT-----KSATT 588

Query: 656 RDNWSSCLOQLIHDLKNEQFKARHHSSTST-----TSSTAKSSSMMSPTTTMNT--PNHH 708
 D S +L + R +TST +SSTA 'S +S + +N+ + + +
 Sbjct: 589 TDERPSDFIRLNSEESVGTSSLRTSQTTSTIVSNDSSSTASIPSOISRISQVNSLLNDYN 648

Query: 709 NSRQTH-----DSMASP---SSSHMKRVSD-----VLPKRRRTSSSPESE 745
 +RQ+H S+ F SSS + + + D + P++ + S+ +S+
 Sbjct: 649 YNRQSHITRVYSGTDDGSSVSIFEDTSSSTKQKIFDQPTTNDCDVMRPRQYSYSAGMKSD 708

Query: 746 IKSISENFKNSIPESSILFRISYNNNNNNTSSSEI---FTLLVEKVWNFDDLIMAINSK 801
 + S+ SS +S N +N + L+V FD+L+ + K
 Sbjct: 709 GSLLPSTKHTSLSSSSTSTSLSVRNTTNUKIRLRLHEVSLVLUVARDITFDELLAKVERK 768

Query: 802 IS--NTHNNNNTSPITKIKYQDEDGDFVVLGSDEDWNVAKE 839
 I + ++KY DEDGDF+ + SDED +A E
 Sbjct: 769 IKLCGILKQAVPFRVRLKYVDEDGDFITITSDEDVLMATE 808

CPU time: 0.26 user secs. 0.04 sys. secs 0.30 total secs.
 Gapped
 Lambda K H
 0.270 0.0470 0.230

Matrix: BLOSUM62
 Gap Penalties: Existence: 11, Extension: 1
 Number of Hits to DB: 10384
 Number of Sequences: 0
 Number of extensions: 671
 Number of successful extensions: 13
 Number of sequences better than 10.0: 1
 Number of HSP's better than 10.0 without gapping: 1
 Number of HSP's successfully gapped in prelim test: 0
 Number of HSP's that attempted gapping in prelim test: 0
 Number of HSP's gapped (non-prelim): 2
 length of query: 834
 length of database: 90,077,593
 effective HSP length: 61
 effective length of query: 773
 effective length of database: 83489227
 effective search space: 64537172471
 effective search space used: 64537172471
 I: 9
 A: 40
 X1: 16 (7.3 bits)
 X2: 128 (49.9 bits)
 X3: 128 (49.9 bits)
 S1: 41 (21.7 bits)
 S2: 73 (32.8 bits)

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Figure 8

5 Sc KLPVIASDDLKVCKKSIVYDFIL (SEQ ID No 25)
 ++PV++SDDL++CKKS+YDF++
 Ca QIPVVSSDDLICKKSIVYDFLI (SEQ ID No 26)

10 Sc = *Saccharomyces cerevisiae*
 Ca = *Candida albicans*

1

Fungal Cdc24's

figure 9

A Fungal Cdc24's

		GEF	PH	% Identity	% Similarity
S.c. Cdc24	1	1-1854			
K.l. Cdc24	1	1-753		53	70
C.a. Cdc24	1	1-3844		92	51
S.p. Cdc24	1	1-872		27	44

B

Guanine nucleotide exchange factor domain of CaCDC24 is homologous to other fungal Cdc24p's

6

Homology of Ste4p binding region

S. c.	174	PACILUNSWKPEQELLETINSEDEK - - VICKASIVYDFTILACMKHPRFENDEELEFTISDUFAN
K. l.	164	PILCILUNSWKPEQELLETINSEDEK - - VICKASIVYDFTILACMKHPRFENDEELEFTISDUFAN
C. a.	150	SEEVILUNSWKPEQELLETINSEDEK - - VICKASIVYDFTILACMKHPRFENDEELEFTISDUFAN
S. p.	119	SICALUNSWKPEQELLETINSEDEK - - VICKASIVYDFTILACMKHPRFENDEELEFTISDUFAN
S. c.	217	SEISQEVETEVWVTEMMNSSTPTEPGSKTKTQIMNAENQRHQPQOSSEKXINSEVQETLEF
K. l.	121	SEISQEVETEVWVTEMMNSSTPTEPGSKTKTQIMNAENQRHQPQOSSEKXINSEVQETLEF
C. a.		NAQDQEVETEVWVTEMMNSSTPTEPGSKTKTQIMNAENQRHQPQOSSEKXINSEVQETLEF
S. p.	179	SEISQEVETEVWVTEMMNSSTPTEPGSKTKTQIMNAENQRHQPQOSSEKXINSEVQETLEF

10

Homology of Bem1p binding region

S.c. 774 -----NTISSEPIPTAEEKUNPODETKAATNSKISNTNNNNI-S-PKEDHVO
K.1. 789 -----DFYATLTSLECHARDMALKREAKLS-----VIAKAVYD
C.a. 746 TPD--VAIETLVEPELESEPIIINAQCHYNDLQHIGITTSNIVADDN-NISERME
S.p. 768 SVRNTTINVILDRIBEVSLVWVADHOTHEPELARVBHTKLGCLDKQAGUPFRVRLKWW

S.c. 822 EDGDEDEVYLGSFEDWVNAKEMIA-----ENTERPLATRIV-----
K.1. 723 EDGDEDEVYLGSFEDWVNAKEMIA-----EDGDEDEVYLGSFEDWVNAKEMIA-----
C.a. 804 DEGDDEDEVYLGSFEDWVNAKEMIA-----EDGDEDEVYLGSFEDWVNAKEMIA-----
S.p. 828 EDGDEDEVYLGSFEDWVNAKEMIA-----EDGDEDEVYLGSFEDWVNAKEMIA-----

Figure 10

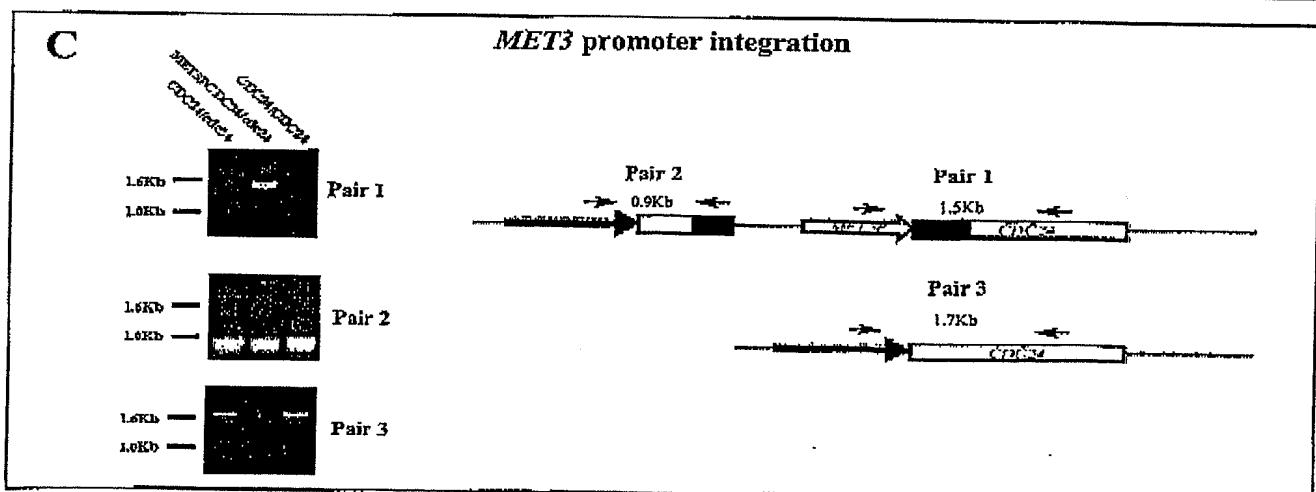
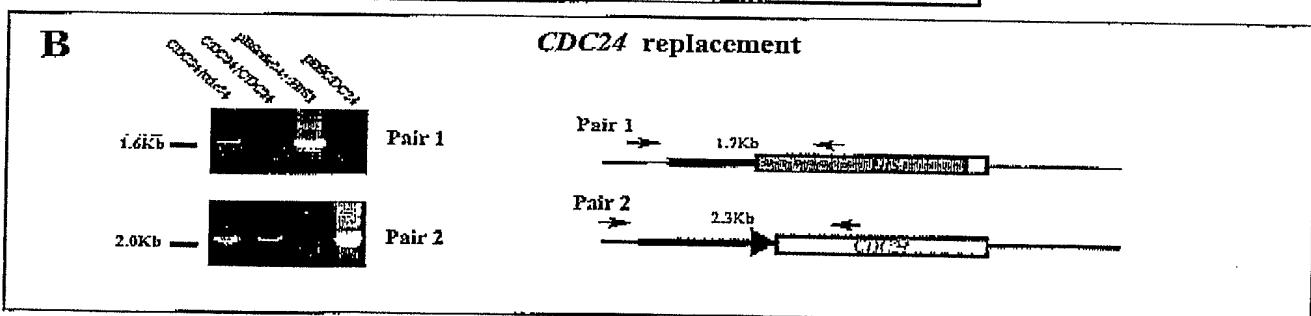
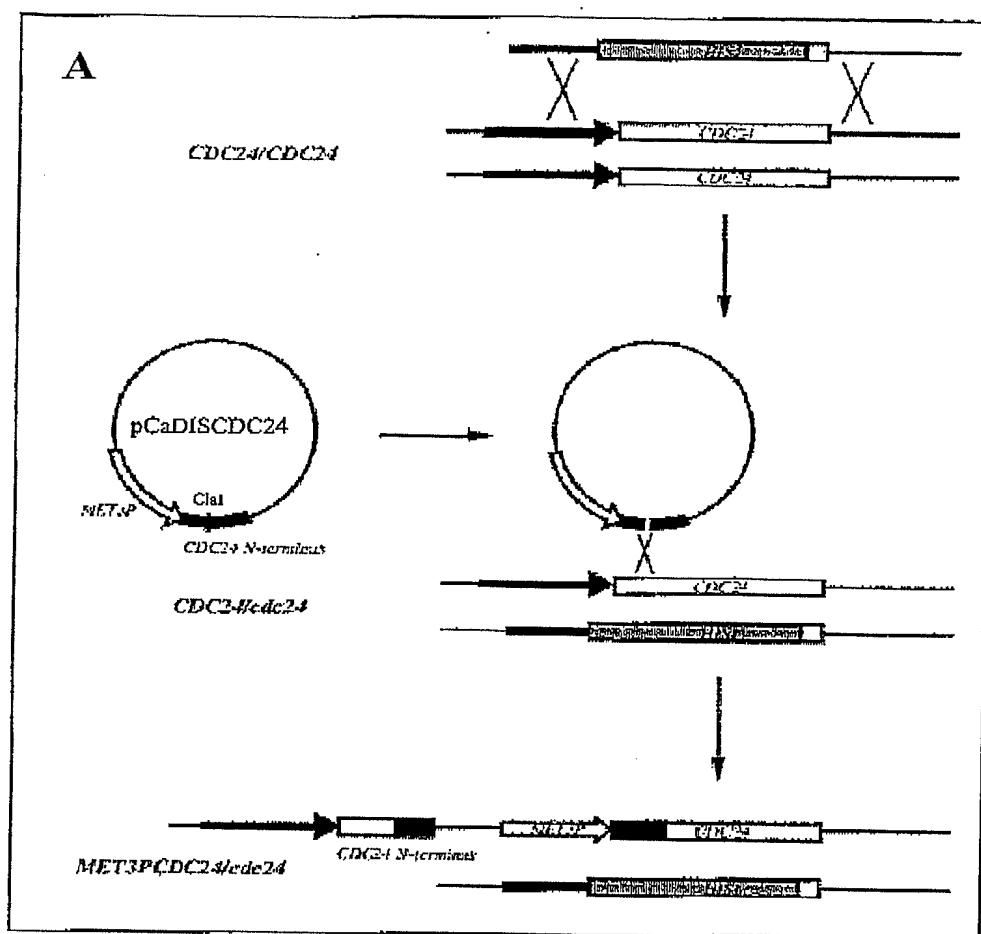
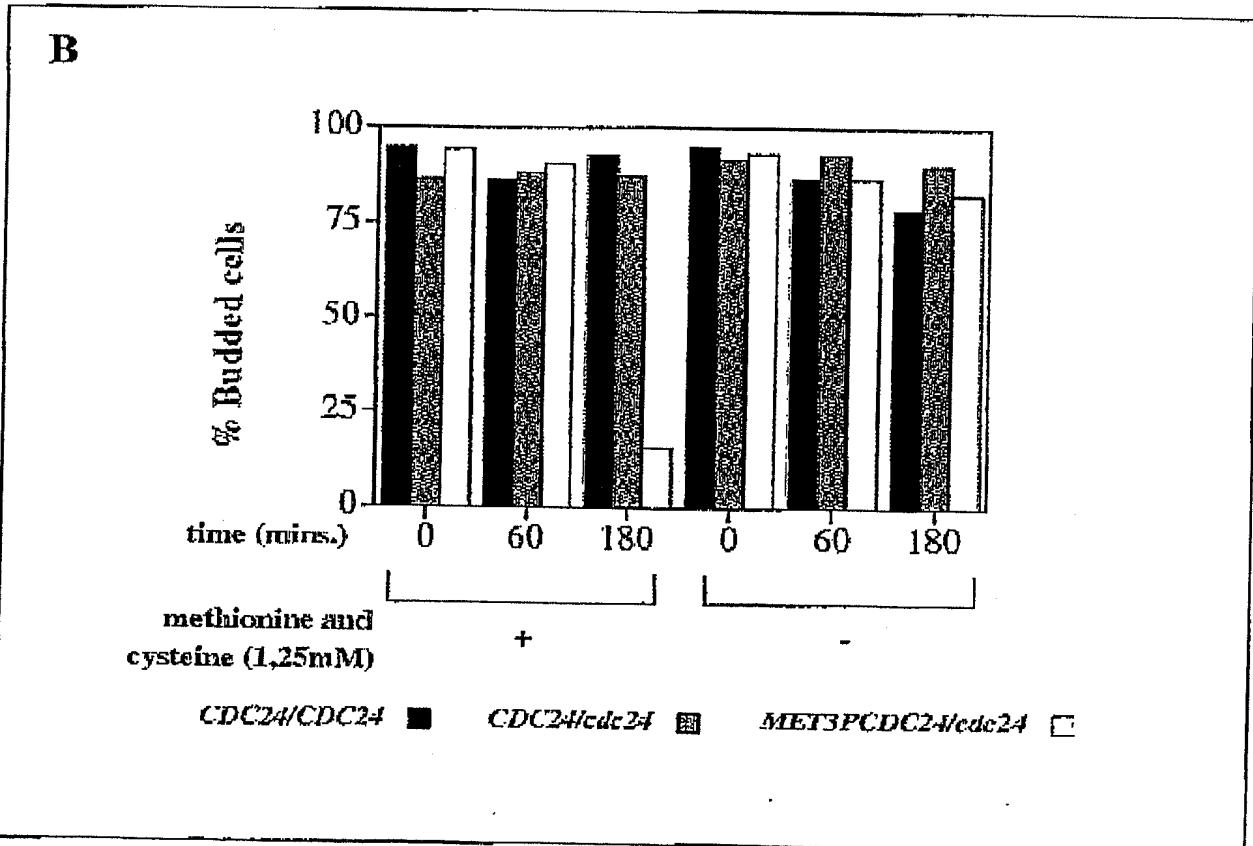
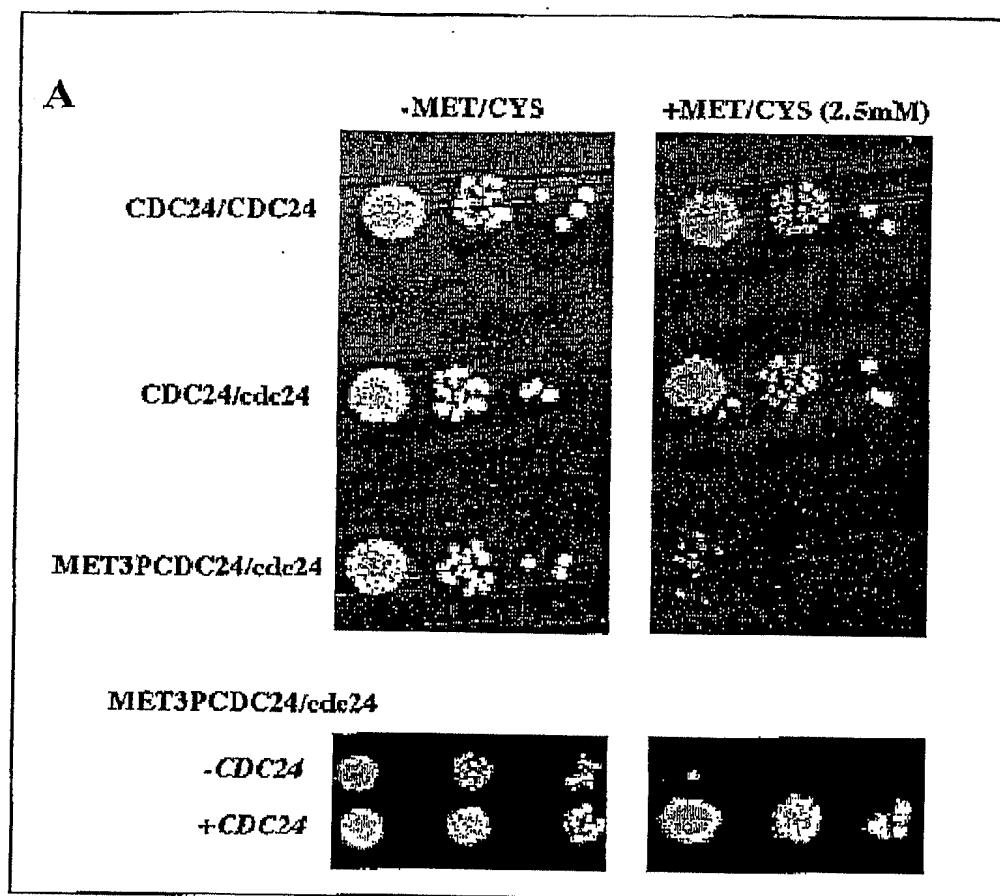


Figure 11



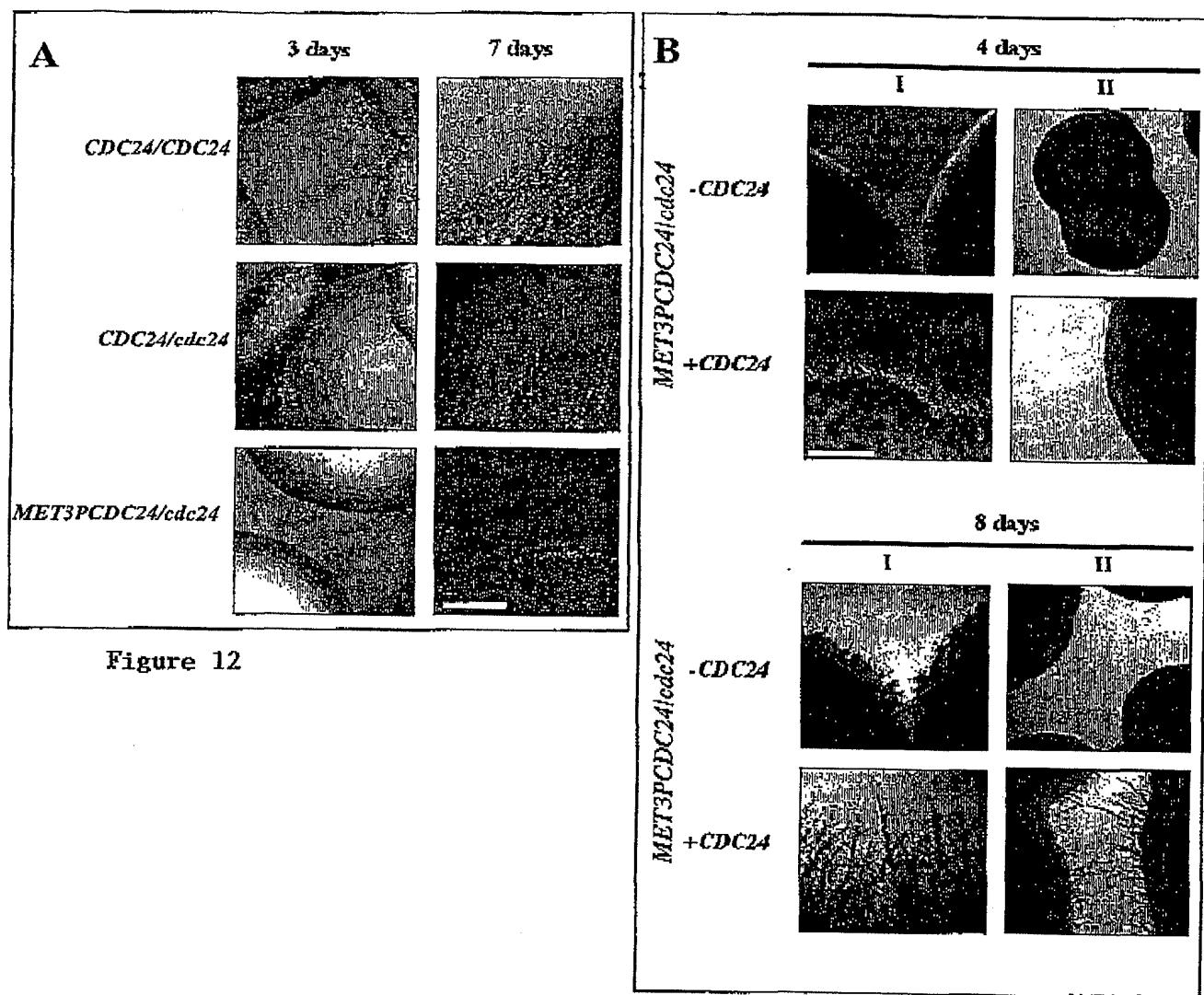
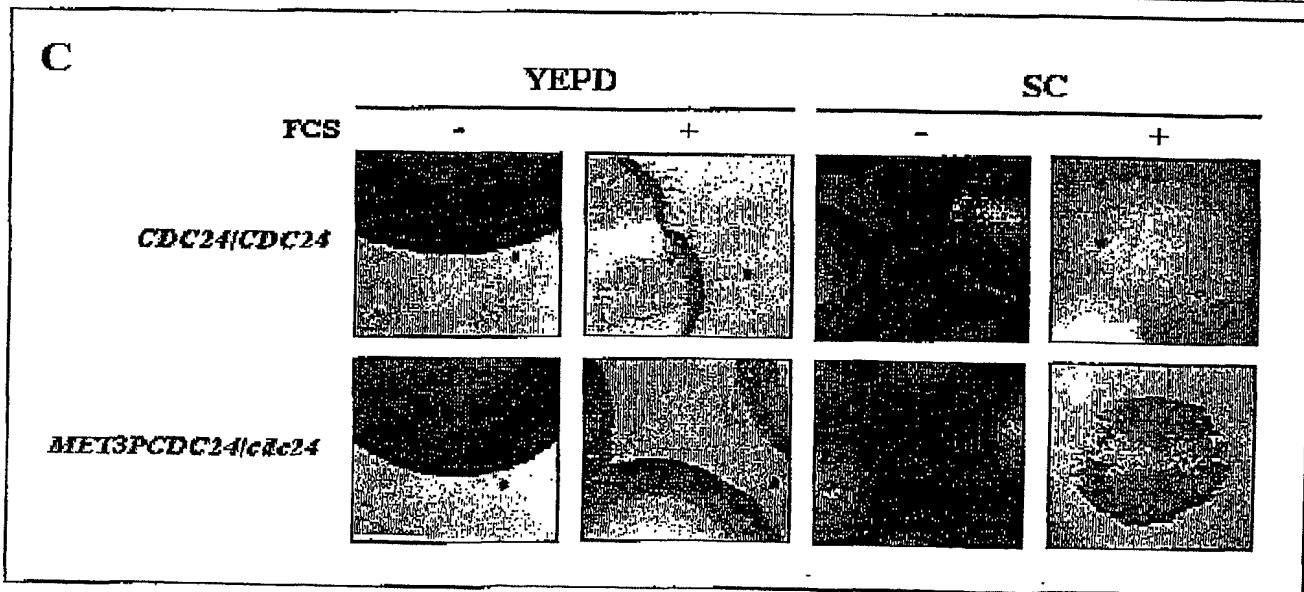


Figure 12



14A

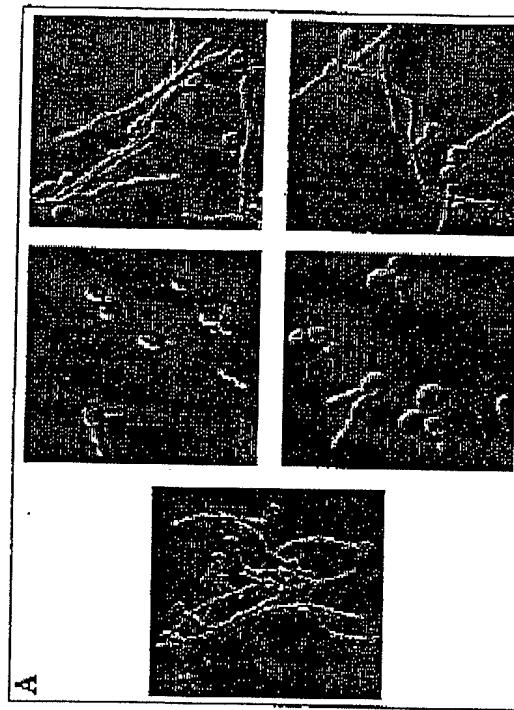
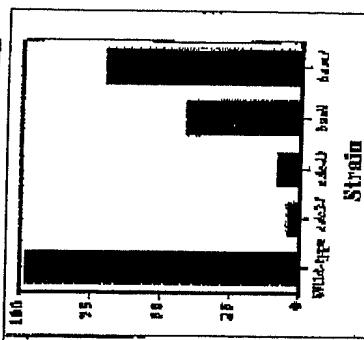


Figure 14B



A

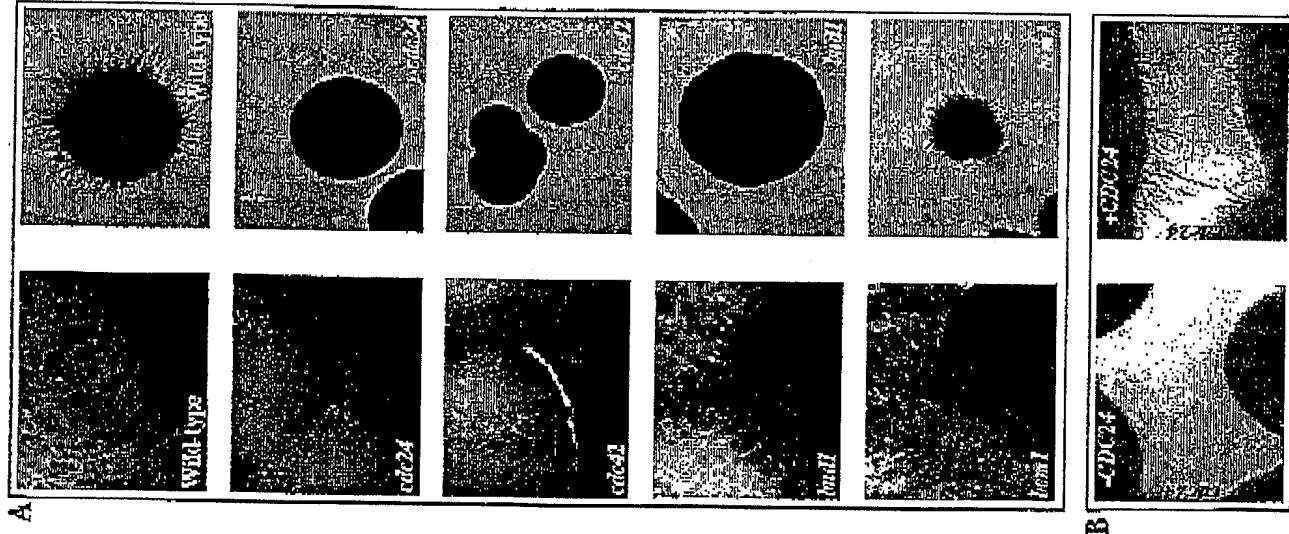


Figure 13

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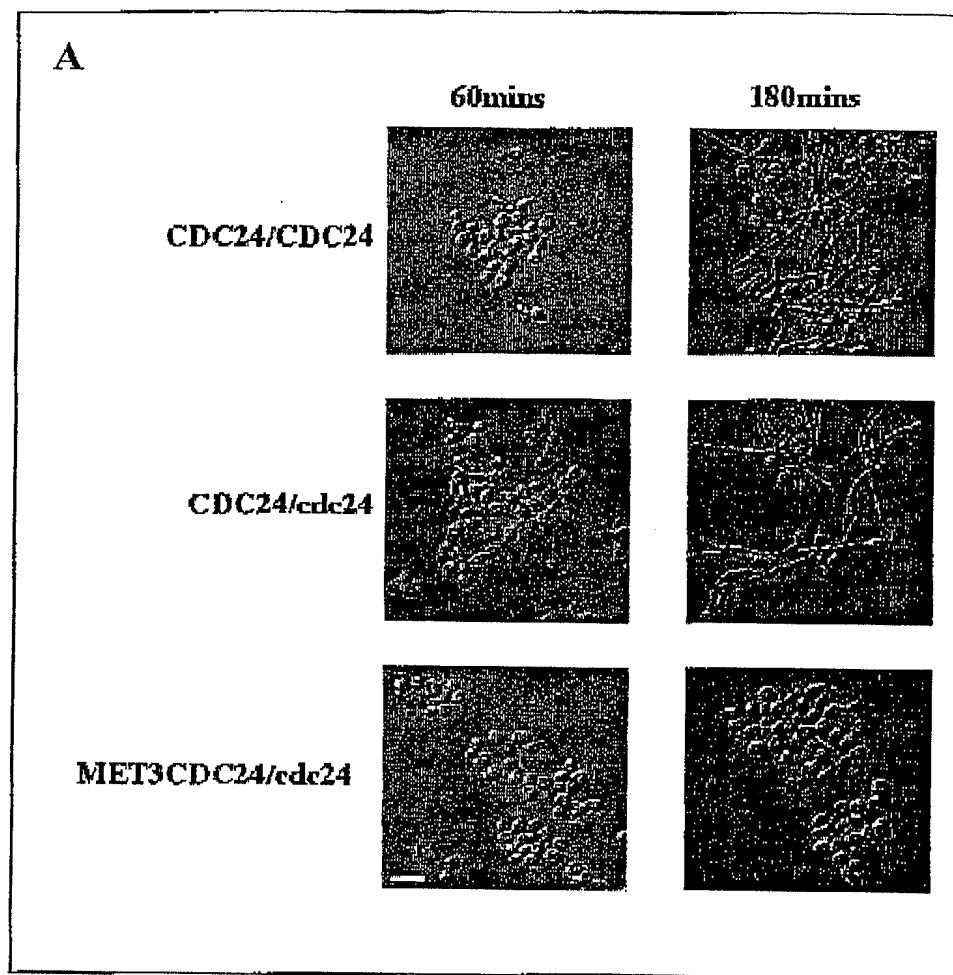
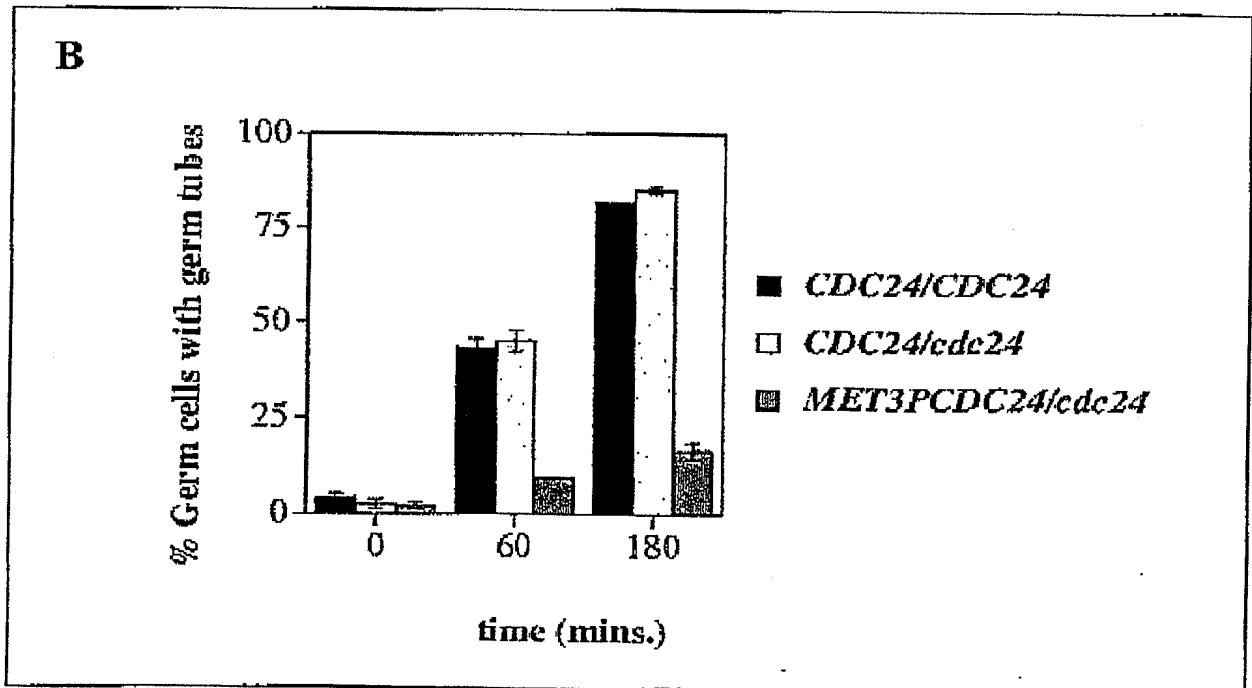
**Figure 15**

Figure 16

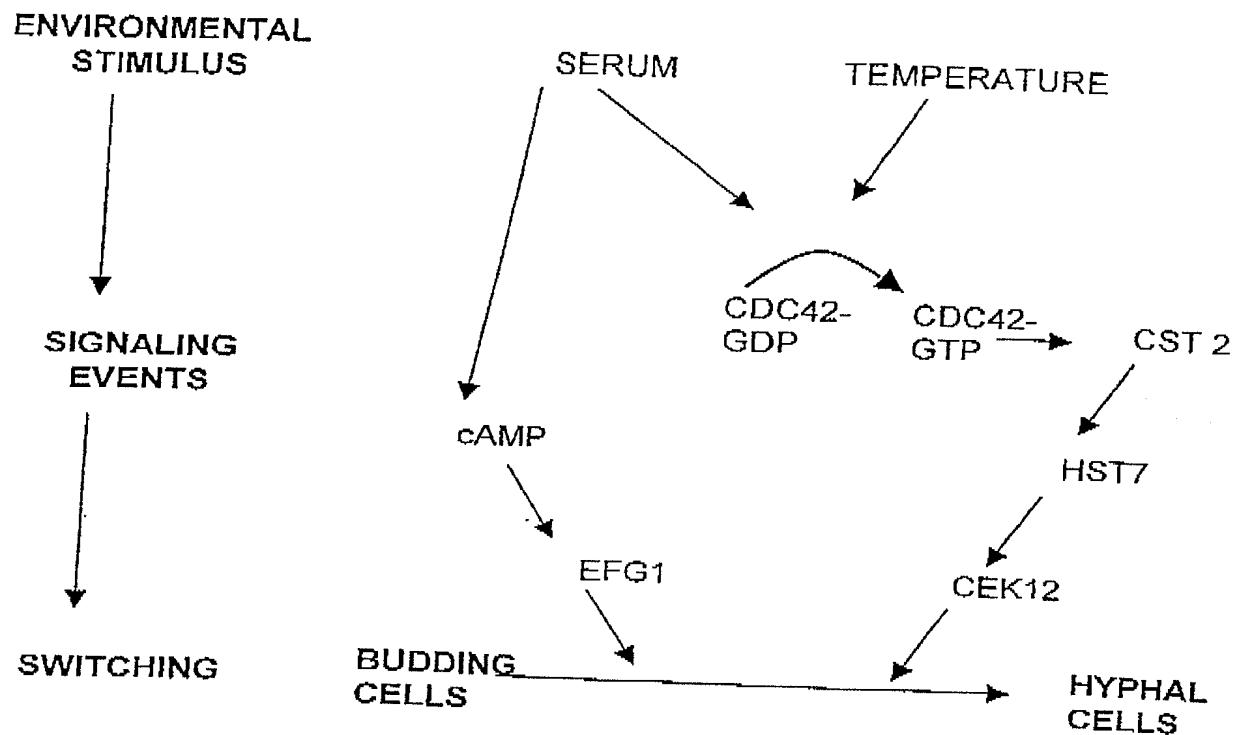


Figure 17

A. *cdc24* (wt)

5

SEQ. I.D. NO:1

DNA:

ccccctctgtatactttcaactctgtgaagccgcaattaaattaccgtaatagcatctgacgattgaaagtctgtaaaaaatccatttatgactt
10 tatattgggctgcaagaaacactttgcatttaacgtgaggagctttcactatatccgacgttttgcactcgacgtcccagctggtcaaagt
gctagaagtagtagaaacgctaataatccagc

SEQ. I.D. NO:2

Protein:

15 PLCILFNSVKPQFKLPVIASDDLKVCKKSIYDFILGCKKHFAFNDEELFTISDVFANSTSQ
LVKVLEVVEVETLMNSS

B. *cdc24-m1*

20 SEQ. I.D. NO:3

DNA:

ccccctctgtatactttcaactctgtgaagccgcaattaaattaccgtaatagcatttgacgattgaaagtctgtaaaaaatccatttatgactt
atattgggctgcaagaaacactttgcatttaacgtgaggagctttcactatatccgacgttttgcactcgacgtcccagctggtcaaagt
gctagaagtagtagaaacgctaataatccagc

25

SEQ. I.D. NO:4

Protein:

PLCILFNSVKPQFKLPVIAFDDLKVCKKSIYDFILGCKKHFAFNDEELFTISDVFANSTSQ
LVKVLEVVEVETLMNSS

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C. *cdc24-m2*

5 SEQ. I.D. NO:5

DNA:

ccccctgtatactttcaactctgtgaagccgcaattaaattaccgtaatagcatctggcgatttgaagtcgtaaaaatccatttgcacit
tatattgggctgcaagaaacactttgcatttaacgatgaggagctttcactatccgacgttttgcacactcgacgtcccagctggcaaagt
gctagaagtagtagaaacgctaattccagc

10

SEQ. I.D. NO:6

Protein:

PLCILFNSVKPQFKLPVIASGDLKVCKKSIYDFILGCKKHFAFNDEELFTISDVFANSTSQ
LVKVLEVVEVETLMNSS

15

D. *cdc24-m3*

SEQ. I.D. NO:7

DNA:

ccccctgtatactttcaactctgtgaagccgcaattaaattaccgtaatagcacctgacgatttgaagtcgtaaaaatccatttgcacit
tatattgggctgcaagaaacactttgcatttaacgatgaggagctttcactatccgacgttttgcacactcgacgtcccagctggcaaagt
gctagaagtagtagaaacgctaattccagc

SEQ. I.D. NO:8

25 Protein:

PLCILFNSVKPQFKLPVIAPDDLKVCKKSIYDFILGCKKHFAFNDEELFTISDVFANSTSQ
LVKVLEVVEVETLMNSS

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SEQ ID NO. 10

STE4 DNA sequence (wild-type)

ATGGCACATCGATGGACTCGATAACGTATTCTAATAATGTCACCCAACAGTATAATACACCCACAGTCTACAGGA
 TACCTCTGCAGTGGAGGAAAGAAATTCAAATATAATAGGGCCAGACAGAGAGTAAACAGCTTCAATGCTAAATAA
 ATTAAGCAAAACACAAGATAACAAGATGCAAGCTTATTCCAGATGGCCACAAAGTTCACCTCGTCAACAAATAAGATC
 5 AACTTAAAGCCAAATATCGTGTGAAAGGCCATAATAATAAACTCAGATTTCCGGGGACTGCTGAGATTCAAACGTAT
 TTEGAGTGCAGTCAGATGGCTTATGCTTATGGGACAGTGCTTCAGGTTAACAGAGCTTCAATTCCATTAGATT
 CTCAAATGGGTCTTCCTGCGCTATTCGCCATCGAGTACTTGGTAGCAACCGCAGGATTAAACATAACTGTACCAATT
 TATAGAGTTCCAAAGAAAACAGAGTAGCGCAAAACGTTGCCTCAATTCAAAGGACATACTGCTATATTCTGACAT
 TGAATTTCAGATAACGCACATAATTGACAGCAAGTGGGATATGACATGTGCCCTGGGTTAACCGAAAGCAAAAGA
 10 GGGTGAGAGAAATTCTGACCAATTAGGTGATGTTTGGCATTAGCTATTCTGAAAGGCCAAACTTAGAAATTCTTCG
 AACACATTGGCTAGCTGGGATCAGACGGGTATACTTACATATGGGATAGCAGATCTCCGTCGGTACAAAGCTTTA
 CGTAAACGAACTGGTACTTAAAGCTGGGATGCTTCTGTTGCTTCTGAAAGGTTAACGAACTGGTACAAAGCTTTA
 TAAATATGTAATTAAAGGTGGACTGTTCTATTGCTACTTTCTGTTCTGAGGTTAACGAACTGGTACCCCTTAC
 CCTACTTTATGGCAGCTAACAGGAGTACAAATCCGGCAATGCCACAAACTTTAAATCAGAGCTTACGCTATCI
 15 AGACAAACCAAGGGTGTGTTCTTAGATTAAAGTGCATCTGGAGATTGTTGAAATTAGGGTCAATGGTGGCAGAGTCAGTGGTGG
 TTGGGTGGGATGTTAAAGGAGAGATTGTTGAAATTAGGGTCAATGGTGGCAGAGTCAGTGGTGGCAGAGTCAGTGGTGG
 CGAGATGGGTTAGCTGTTGACAGGTTCAATGGGACTCAACGATGAAATAGGTCTCCAGGTTTCAGAT

20

SEQ ID NO. 11

Ste4 Protein sequence (wild-type)

MAHQMDSTIYNNVTCOYIQPQLQDISAVEEEIQNKIEAARQESKQLHAQINKAKHKTQDASLFQMANKVTSLTQNKEI
 LKPNIVLKGERNNKISDERWSRDSKRILSASQDGFMILWDSASCLKQNAIFLDQSQWVLSCATCPESTLVAASAGLNNNCTI
 25 RVSKEKRVQAQXVASTIFKQHTCYISDIEFTDNAHILTAGDMTCALWDIPKAKRVREYSDHLGCVLALATFEEPNLENSI
 TTASCGSDGYTYIWDERSPSAVQSFYVNDSDINALRFFKDGMSIVAGSDNGAINMYDLRSDCSIATFSLFQGYEEATPT
 TYMAANMEYNTRQSPQTLKSTSSSYLDNQGVVSLDFSASGRLMYSCTYDIGCVVWDVLKGEIVGKLEGHGRVTGVRES
 DGLAVCTGSWDSTMKIWSPGYQ

30

SEQ ID NO. 12

ste4-o15 DNA sequence (mutant)

ATGGCACATCGATGGACTCGATAACGTATTCTAATATGTCACCCAACAGTATAATACACCCACAGTCTACAGGA
 35 TACCTCTGCAGTGGAGGAAAGAAATTCAAATATAATAGGGCCAGACAGAGAGTAAACAGCTTCAATGCTAAATTT
 ATTAAGCAAAACACAAGATGCAAGCTTATTCCAGATGGCCACAAACAGCTTACTTGTGAAAGGAT
 AACTTAAAGCCAAATATCGTGTGAAAGGCCATAATAATAAACTCAGATTTCCGGGGACTGAGATTCAAACGTAT

5 TTTGAGTGCAAGTCAAGATGGCTTATGCTTATGGACAGTGCTCAGGTTAAACAGAACGCTATTCCATTAGAT
 CTCATGGGTCTTCCTGCCTATTCGCCATCGAGTACCTGGTAGCAAGCGCAGGATTACATAACTGTACCAT
 TATAGAGTTCGAAAGAACAGAGTAGCGCAAAACGTTGCGTCAATTCAAAGGACATACTGCTATATTCTGACA
 TGAATTACAGATAACGCACATATATTGACAGCAACTGGGATATGACATGTGCCTTGTGGGATATCCGAAAGCAAAG
 10 5 GGGTGAGAGGATATTCTGACCATTAGGTATGTTGGCATTAGCTATTCTGAAGAGCCAACTTAGAAAATTCTTC
 AACACATTGCTAGCTGTTGATCAGACGGGTATACTTACATAAGGATAGCAGATCTCCGTCCGCTGTACAAAGCTTT
 CGTTAACGATAGTGTATTAATGCACTCGTTTTCAAAGACGGGATGTCGATTGTCAGGAAGTGACAATGGTGC
 TAAATATGATGATTAAGGTGCGACTGTTCTATTGCTACTTTTCTCTTTGAGGTTATGAAGAACGTAACCCCTAC
 CCTACTTATGGCAGCTAACATGGAGTACAATACCGCGCAATGCCACAAACTTAAATCAGCTCAAGCTATC
 15 10 AGACAACCAAGGCCTGTTCTTAGATTTAGTGCATCTGGAAAGATTGATGTAECTCATGCTATACAGACATTGGTTGT
 TTGTGTGGGATGTTAAAGGAGAGATTGTTGAAAATTAGAAGGTATGGCAGAGTCACTGGTGTGGCAGGTTATG
 CGGATGGGTTAGCTGTATGTCACGGTTCATGGGACTCSECCATGAAATATGCTCCAGGTTATGATC
 SEQ ID No. 13

15 Ste4-o15 Protein sequence (mutant)

MAHQMDSITYSNNTQOYIQPQLQDISAVEEEIQNKIEARQESKQLHAQINKAKHKGDSLQMANVTSLTKNK
 LKPNTIVLKGNNKISDFRWSRDSKRILSASQDFMLIWDASGLKQNAIPLO\$QWVLSCAISPSSTLVASAGLNNNCT
 RVSKENRVAQNVASIFKGHTCYISDIEFTDNAHILTDASGDMTCALNDIPKAKRVRGYSQHLDVLAALAIPEEPNLENS.
 20 20 TFASCSDGTYTIIWDSRSPSAVQSFYVNDSDINALRFFKDGMMSIVAGSDNGTINMYDLRSDCIAATFSLFRGYEERTP
 TYMAANMEYNTAQSPQTLKSTSSSYLDNQGVVSLDFASGRLMYSCYTDJGCVVWDVLKGEIVGKLEGHGRVTGVRS:
 DGLAVCTGSDSTMKIWSPGYQ

SEQ ID NO. 14

25 ste4-o17 DNA sequence (mutant)

ATGGCACATCAGATGGACTCGATAACGTAATTCTAAATGTACCCAACAGTATATAACACCAAGTCTACAGGA
 TATCTCTGCAGTGGAGGAAGAAATTCAAATAAAATAGAGGCCGCCAGACAAGACAGTAAACAGCTCATGCTCATATA
 ATAAAGCAAAACACAAGATAACAGATGCAAGCTTATTCCAGATGGCCAAACAAAGTTACTTCGTTGCCAAATAAGAT
 30 30 AACTAAAGCCAAATATCGTGTGAAAGGCCATAATAATAAAATCTCAGATTTCGGTGGAGTCGAGATTCAAAACGTA
 TTTGAGTGCAAGTCAAGATGGCTTATGCTTATATGGGACAGTGCTTCAGGTTAAACAGAACGCTATTCCATTAGAT
 CTCATGGGTCTTCCTGCCTATTCGCCATCGAGTACTTGGTAGCAAGCGCAGGATTACATAACTGTACCAT
 TATAGAGTTGAGAACAGAGTAGCGCAAAACGTTGCGTCAATTCAAGGACATACTTGCTATATTCTGACA
 TGAATTACAGATAACGCACATATATTGACAGCAAGTGGGGATATGACATGTGCCTTGTGGGATATACCGAAAGCAAAG
 35 35 GGGTGAGAGAATATTCTGACCATTAGGTATGTTGGCATTAGCTATTCTGAAGAGCCAAACTTAGAAAATTCTTC
 AACACATTGCTAGCTGCGATCAGACGGGTATACTTACATATGGGATAGCAGATCTCCGTCCGCTGTACAAAGCTTT
 CGTTAACGATAGTGTATTAATGCACTTCGTTTTCAAAGACGGGATGTCGATTGTCAGGAAGTGACAATGGTGC

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TAATATGATTAAAGTCGGACTGTTCTATTGCTACTTTCTCTTTCGAGGTTATGAAGAACGTACCCCTAC
 CCTACTTATATGGCAGCTAACATGGAGTACAATACCGCGCAATGCCACAAACTTAAAATCAACAGCTCAAGCTATCT
 AGACAACCAAGGCCTGTTCTTAGATTTAGTGCATCTGGAAAGATTGATGTAACATGCTATACAGACATTGGTTGTC
 5 CCAGATGGGTTAGCTGTATGTACAGGTTCATGGGACTCAACCATGAAAATATGGTCTCCAGGTTATCAATAG

SEQ ID No. 15

Ste4-o17 Protein sequence (mutant)

10 MAHQMDSDITYNNVTQQYIQPQSLQDISAVEEEIQNKIEAARQESKQLHAQINKAKHKGQDASLFCMANKVTSLTKNKIP
 LKPNIVLKGHHNKISDFRWSRDSKRILSASQDGFMILWDSASGLKQNAIPLDQSQWVLSCAISPSTLVASAGLNNNCTIR
 RVSKENRVAQNVASIFKGHTCYISDIEFTDNAHILTA3GDMTCALWDIPKPKRVRREYSDHLDVIALRIPEEPNLENSST
 TFASCSDGTYIWDSSRSPSAVQSFYVNDSDINALRFFKDGMSTIVAGSDNGAINMYDLRSDCSIAESTLFRGYEERTPTI
 15 TYMAANMEYNTAQSPQTLKSTSSSYLDNQAV5LDFSAASGRMLMYSCYTDIGCVVWDVLKGEIVGKLEGHGGRVTGVRSS
 DGLAVCTGSWDSTMKIWSPGYQ

SEQ. I.D. No:16 refers to the epitope sequence "Tyr Pro Tyr Asp Val Pro Asp Tyr Ala".

SEQ. I.D. No:17 refers to TEV protease recognition sequence "Gln Asn Leu Tyr Phe Gln Gly"

SEQ. I.D. No:18 refers to peptide sequence "QFKLPVIAFDDLKVCKKSI".

SEQ. I.D. No:19 refers to peptide sequence "QFKLPVIASGDLKVCKKSI".

SEQ. I.D. No:20 refers to peptide sequence "QFKLPVIAPDDLKVCKKSI".

SEQ. I.D. No:21 refers to peptide sequence "QFKLPVIASDDLKVCKKSI".

SEQ. I.D. No:22 refers to peptide sequence "QYEFDVILSPELKVQMKTI".